

*Research article*

## CARDIAC TROPONIN-I CONCENTRATION IN WEANED CALVES WITH BOVINE RESPIRATORY DISEASE

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The aim of this study was to evaluate the serum cardiac troponin I (cTnI) concentrations in weaned calves with moderate and severe bovine respiratory disease. Eighteen healthy weaned calves (group I), 18 weaned calves with moderate bovine respiratory disease (group II) and 18 weaned calves with severe bovine respiratory disease (group III) were included in the study. Clinical signs and severity of respiratory disease according to clinical index scores were determined. Blood cell counts and cardiac biomarkers, such as serum cTnI concentrations and creatine kinase-myocardial band (CK-MB), and lactate dehydrogenase (LDH) activities were determined in all groups. Temperature and respiratory and pulse rates were significantly increased in calves with moderate and severe bovine respiratory disease compared to healthy calves ( $P < 0.05$ ). Serum cTnI concentrations and LDH activities were significantly increased in the calves with severe bovine respiratory disease compared to healthy calves and calves with moderate bovine respiratory disease ( $P < 0.05$ ). A significant increase in white blood cell counts in calves with moderate and severe bovine respiratory disease and lymphopenia and neutrophilia in calves with severe bovine respiratory disease were also found. The study suggests that severe bovine respiratory disease causes increased serum cTnI concentrations in weaned calves.

**Key words:** bovine respiratory disease, cardiac biomarkers, hematologic parameters, calves.

### INTRODUCTION

Bovine respiratory disease (BRD) causes important morbidity, mortality and economic losses in beef and dairy cattle [1]. BRD is caused by stress including weaning, commingling of cattle from various sources, poorly ventilated areas, overcrowding,

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environmental temperature and shipping [1,2]. BRD may also result from exposure to multiple pathogens, especially viral pathogens such as bovine herpes virus-1, bovine respiratory syncytial virus, parainfluenza-3, bovine viral diarrhoea virus, adenoviruses (types 3 and 7), rhinoviruses, and coronaviruses. Bacterial pathogens such as *M. haemolytica*, *P. multocida*, *Mycoplasma* spp., *H. somni*, and *A. pyogenes* may also cause BRD [3,4].

Of these pathogenic bacteria, *H. somni* has a tendency to cause heart damage, and a septic thrombus in the caudal vena cava can create bronchopneumonia and endocarditis [3]. Fever, hypoxia, septicemia, complement and coagulation initiation, increased acute phase proteins, exotoxin and endotoxin production, lung abscesses, consolidation, and vasculitis may occur in BRD [3,5-7] and could lead to myocardial injury [3,5,6,8].

Cardiac troponin is a myofibrillar protein that controls contractions of the heart; it has two forms, cardiac troponin I (cTnI) and cardiac troponin T (cTnT) that are both used in the diagnosis of cardiac injury in humans and animals. As long as the cardiac injury continues, cardiac troponin concentrations remain elevated. Cardiac troponin is considered the gold standard biomarker of cardiac injury because of its high tissue specificity, diagnostic sensitivity, low basal blood concentration, rapid release, and persistence in the blood [9-12].

Troponin analyses are more sensitive and specific than creatine kinase-myocardial band (CK-MB) and lactate dehydrogenase (LDH) assays. Also, CK-MB expression is not limited to the heart and is also expressed in other tissues such as skeletal muscle and the gastrointestinal tract. Therefore, compared to CK-MB, cardiac troponin is chosen for the specific detection of cardiac injury [5]. Additionally, cTnI, not cTnT, is the essential indicator for detecting and quantifying an active myocardial injury in cattle [11]. Several studies have reported that cardiac troponin concentrations are increased in cases of community-acquired pneumonia in humans [6], and chronic suppurative pneumonia in cattle [13].

Cardiac troponin I concentrations have been reported in various diseases in cattle [14-16] but not in BRD. Therefore, the present study was aimed to evaluate the serum cardiac troponin I concentrations in weaned calves with moderate and severe BRD.

## MATERIAL AND METHODS

### **Animals**

We used 18 healthy weaned calves (group I) and 18 weaned calves with moderate BRD (group II) and 18 weaned calves with severe BRD (group III). All calves were 4 to 8 months old. They were the Swiss Brown breed and were supplied from weaned calves housed in private farms in Erzurum province of the eastern region of Turkey. Moderate and severe respiratory disease in weaned calves was determined according to the clinical index score.

### **Clinical examination**

Clinical examination signs included rectal temperature, pulse and respiratory rate (per minute), cough, dyspnoea, nasal discharge, and respiratory sounds. Clinical parameters, observation grading and weighted numerical score included demeanour (normal = 2, depressed = 4, moribund = 8); appetite (normal = 2, depressed = 4, anorexic = 8); rectal temperature (<39.5 °C = 4, >39.5 °C but <40.5 °C = 8, >40.5 °C = 12); respiratory rate (per minute) (<35 = 3, 35-50 = 6, >50 = 9); type of respiration (normal = 1, dyspnoeic = 2); cough (absent = 1, present = 2); and nasal discharge (absent = 1, present = 2). Clinical index scores were calculated by adding up the weighted numerical scores from the animal's observation grade divided by the total weighted clinical score of 79 [17].

### **Hematologic and biochemical assay**

Hematologic parameters including red blood cells (RBCs), haemoglobin, haematocrit values, white blood cells (WBCs), the percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils, mean corpuscular volume (MCV), and platelets (PLT) were determined for each group. Also analyzed for each group were various cardiac biomarkers including cTnI concentrations and CK-MB and LDH activities. For analysis of the hematologic parameters and cardiac biomarkers, blood samples were collected by direct puncture of the *vena jugularis* of all calves into EDTA vacutainers (BD Vacutainer®, K2E 3.6 mg, BD-Plymouth, UK) and plain tubes (Aysset® Tube, Clot Activator & Gel (ZS), Turkey) and serum samples were stored - 20 °C until further analysis. Blood cell counts were measured with a haematology analyser specific for cattle (Abacus Junior Vet 5-Diatron GmbH, Austria). The serum cTnI concentrations were determined by a commercial immunoassay system according to the one-step sandwich method (Unicel Beckman Coulter Access II, USA). Troponin I sequence homology between humans and cattle is > 96% [18], thus this assay may be reliably used in calves. The immunoassay system can measure troponin in a range of 0.01-100 ng/mL. Serum enzymes such as CK-MB (CK IFCC method plus Immunoinhibition), LDH (L-P'a IFCC) activities were determined by a biochemistry autoanalyser (Beckman Coulter, AU5800, USA) using commercial enzyme kits.

### **Analysis of viruses**

Nasal swap specimens obtained from all the animals were kept at -80 °C until further analysis. In the thawed nasal swap specimens, viral nucleic acids were extracted by a viral nucleic acid extraction kit (Vivantis, Malaysia). After viral nucleic acid extraction process, nucleic acids, including RNA, the bovine respiratory syncytial virus and parainfluenza-3, were converted to complementary DNA using RevertAid First Strand cDNA Synthesis Kit (Cat No: K1622, Thermo Scientific). The positivites for the viral agents of BRD were determined by the polymerase chain reaction (PCR) process [19-21].

### Statistical analysis

Statistical analysis was performed between groups I, II and III. Tests of normality were made according to Kolmogorov-Smirnov test. If there was normal distribution for variables, an analysis of variance (ANOVA) was used for the determination of significance between groups. This was followed by a Dunnett's T3 test of multiple comparisons if variances had no homogeneity or a post hoc Tukey test if variances of groups had homogeneity. If there was no normal distribution, the Kruskal Wallis test was used for the determination of significance between all groups, followed by a multiple comparisons test to determine for which group the significance is derived from. A p-value of  $< 0.05$  was considered significant.

## RESULTS

### Clinical signs

Temperature and respiratory and pulse rates were significantly increased in the moderately and severely diseased calves compared with healthy calves ( $P < 0.05$ ) as shown in Table 1. The calves with moderate and severe respiratory disease had clinical signs such as coughing, nasal discharge (serous, mucous or mucopurulent), dyspnoea, adventitious lung sounds, decreased appetite or anorexia, and a depressed appearance.

**Table 1.** Clinical examination parameters in healthy calves and in calves with bovine respiratory disease

Clinical Parameters	Group I (n=18)	Group II (n=18)	Group III (n=18)
T (°C)	38.20 (38.00-38.70) 38.24±0.05	39.50 (38.00-39.50) <sup>b</sup> 39.16±0.13 <sup>b</sup>	40.15 (39.70-41.00) <sup>a</sup> 40.29±0.10 <sup>a</sup>
R (breaths/min)	20 (16-22) 19.11±0.46	49 (32-50) <sup>d</sup> 45.33±1.64 <sup>d</sup>	76 (56-112) <sup>c</sup> 77.83±4.28 <sup>c</sup>
P (beats/min)	90.11 ± 0.59 <sup>e</sup>	107 ± 5.33	116.22 ± 6.62
Clinical index score		0.34	0.45

<sup>a</sup>  $P < 0.05$  versus group I and group II; <sup>b</sup>  $P < 0.05$  versus group I; <sup>c</sup>  $P < 0.05$  versus group I and group II;

<sup>d</sup>  $P < 0.05$  versus group I; <sup>e</sup>  $P < 0.05$  versus group II and group III

Group I: Healthy calves; Group II: Moderately diseased calves; Group III: Severely diseased calves; T: Temperature; R: Respiratory rate (per min); P: Pulse rate (per min)

All the values are expressed as median (minimum – maximum) and mean ± SEM for 18 weaned calves per group.

### The determined viral agents

The PCR positivity rates for bovine herpes virus 1, bovine respiratory syncytial virus, and parainfluenza-3 were 36.1%, 38.9%, and 25%, respectively.

### The results of serum biochemistry and hematologic parameters

Serum biochemistry and hematologic parameters are given in Table 2. Serum cTnI concentrations and LDH activities were significantly increased in the severely diseased calves compared with the healthy and moderately diseased calves ( $P < 0.05$ ). There was no significant difference for the serum CK-MB activity between groups. WBC counts were significantly increased in the moderately and severely diseased calves compared with the healthy calves ( $P < 0.05$ ). The percentage of lymphocytes was significantly decreased in the severely diseased calves compared with the healthy and moderately diseased calves ( $P < 0.05$ ). The percentage of neutrophils was significantly increased in the severely diseased calves compared with the healthy and moderately diseased calves ( $P < 0.05$ ). There were no significant differences for haematocrit, MCV, PLT and the percentage of monocytes, eosinophils and basophils between groups. RBC and haemoglobin levels were significantly increased in the severely diseased calves compared with the healthy and moderately diseased calves ( $P < 0.05$ ).

**Table 2.** Cardiac biomarkers and hematologic parameters in healthy calves and calves with bovine respiratory disease

Parameters	Group I (n=18)	Group II (n=18)	Group III (n=18)
cTnI (ng/mL)	0.02 (0.01-0.04) 0.0228±0.002	0.02 (0.01-0.04) 0.026±0.002	0.1 (0.04-2.84) <sup>a</sup> 0.308±0.156 <sup>a</sup>
CK-MB (U/L)	206.50 (163-492) 232.22±17.54	225 (104-651) 309.67±41.05	300 (105-1221) 427.11±26.28
LDH (U/L)	658.61 ± 28.97	677.94 ± 62.16	905.39 ± 54.17 <sup>b</sup>
WBCs (x10 <sup>3</sup> /μL)	6.88 ± 0.21	10.69 ± 0.36 <sup>d</sup>	20.52 ± 2.0 <sup>e</sup>
Lymphocytes (%)	60.82 ± 2.58	58.92 ± 3.31	41.57 ± 3.49 <sup>e</sup>
Neutrophils (%)	32.11 ± 2.47	34.54 ± 3.23	53.08 ± 3.86 <sup>f</sup>
Monocytes (%)	1.05 (0.50 - 9.50) 3.08±0.78	1.80 (0.50 - 9.90) 4.07±0.897	1.10 (0.60 - 9.10) 3.36±0.836
Eosinophils (%)	1.75 (0.50 - 14.30) 3.88±1.086	1.90 (0.40 - 6.40) 2.39±0.44	1.25 (0.40 - 9.80) 1.91±0.51
Basophils (%)	0.10 (0.00 - 0.50) 0.12±0.034	0.10 (0.00 - 0.30) 0.08±0.019	0.05 (0.00 - 0.40) 0.08±0.026
RBCs (x10 <sup>6</sup> /μL)	8.37 ± 0.47	8.28 ± 0.38	9.97 ± 0.52 <sup>g</sup>
Haemoglobin (g/dL)	9.82 ± 0.32	9.05 ± 0.37	10.52 ± 0.48 <sup>h</sup>
Haematocrit (%)	33.11 ± 1.29	29.26 ± 1.25	33.01 ± 1.64
MCV (fL)	42.55 ± 3.53	36.77 ± 1.64	34 ± 1.73
PLT (x10 <sup>3</sup> /μL)	372.27 ± 33.44	376.44 ± 20.86	385 ± 47.99

<sup>a</sup>  $P < 0.05$  versus group I and group II; <sup>b</sup>  $P < 0.05$  versus group I and group II; <sup>c</sup>  $P < 0.05$  versus group I and group II; <sup>d</sup>  $P < 0.05$  versus group I and group III; <sup>e</sup>  $P < 0.05$  versus group I and group II; <sup>f</sup>  $P < 0.05$  versus group I and group II; <sup>g</sup>  $P < 0.05$  versus group I and group II; <sup>h</sup>  $P < 0.05$  versus group I and group II  
Group I: Healthy calves; Group II: Moderately diseased calves; Group III: Severely diseased calves; cTnI: Cardiac troponin I; CK-MB: Creatine kinase-myocardial band; LDH: Lactate dehydrogenase; WBC: White blood cells; RBC: Red blood cells; MCV: Mean corpuscular volume; PLT: Platelet.

All the values are expressed as median (minimum – maximum) and mean ± SEM for 18 weaned calves per group.

## DISCUSSION

This study assessed the enzymes cTn I, CK-MB, and LDH for the detection of possible myocardial damage secondary to moderate and severe respiratory disease in weaned calves. Increased serum cTnI concentrations were determined in the weaned calves with severe BRD. However, there was no increase for the serum cTnI concentration in the moderately diseased calves.

Cardiac troponin assays designed for human use are a sensitive and specific marker for the determination of myocardial degeneration and necrosis in animals [15,22]. Some biomarkers, such as CK-MB and LDH, do not come close to the cardiac troponin biomarker in the evaluation of myocardial injury. LDH and CK-MB, which are found in skeletal muscle and heart, do not have any tissue sensitivity and specificity. Additionally, their cardiac activities are lower than the total enzyme activities [10,12]. In the study, cTnI concentrations and LDH activities were significantly increased in the severely diseased calves compared with the healthy and moderately diseased calves. Furthermore, there was no significant difference for CK-MB between the studied groups. This study suggests that myocardial myocytes might be affected because of increased serum cTnI in severe BRD in weaned calves.

The viral and bacterial agents of pneumonia can cause myocarditis in humans [23]. Sporadic myocarditis or myocardial necrosis in patients with severe influenza B virus [24,25] and significant frequency of bacterial pneumonia in patients with cardiac injury [16] have also been reported. Hoar *et al.* [26] have reported myocarditis findings consistent with *H. somnus* in calves with bronchopneumonia that died after the treatment period. In the present study, we detected the PCR positivity rate for bovine herpes virus 1 as 36.1%, bovine respiratory syncytial virus as 38.9%, and parainfluenza-3 as 25%, from the viral agents of BRD in the diseased groups.

Increased serum cardiac troponin levels have been found in a wide variety of clinical conditions such as myocarditis, non-ischemic cardiac disorders, atrial fibrillation, hypovolemia, sepsis, endotoxemia [27], ruminal acidosis [28], theileriosis [14] and *M. bovis*-induced pneumonia [29]. Toxic effects from bacterial endotoxins, cardiac depressant effects from cytokines, cardiac oxygen demands exceeding supplies, which are developed with inflammation, ventilation-perfusion mismatches in acute pneumonia, depression of myocardial contractility, catecholamine release, and tachycardia may cause increased serum cardiac troponin levels [6,30]. In these conditions increased serum cardiac troponin levels are linked with the release of free cytosolic or structurally bound troponin [12]. In this study, serum cTnI concentrations were increased in the severely diseased calves more than in the moderately diseased calves. Therefore, inflammation, tachycardia, hypoxia, and cytokines involved in the pathogenesis of BRD [1] may have been more severe in the severely diseased calves than in the moderately diseased calves; this is consistent with the clinical index scores and significantly increased WBC counts.

A greater severity of pneumonia can lead to cardiac complications [6]. The occurrence of tachycardia caused by hypoxemia in pneumonia cases is expressed by an increase in the sympathetic activity, causing an increased myocardial oxygen demand [26]. Moammar et al. [31] have noted that decreased blood oxygen levels may play a role in acute myocardial damage in patients with community-acquired pneumonia. Furthermore, Corrales-Medina et al. [26] reported that cardiac arrhythmias occur as a complication of acute pneumonia. In the present study, increased pulse rates were found in the moderately and severely diseased calves but respiratory rates were significantly increased only in calves with severe BRD compared to calves with moderate BRD.

Complete blood cells are variable and nonspecific in BRD. Hematology may show neutrophilia, neutropenia or be normal in BRD and may be associated with a different etiology and dependent upon whether the disease is in the acute or chronic stage [3]. In this study, neutrophilia and lymphopenia were found in the severely diseased calves. There were no significant differences for haematocrit, MCV, PLT or the percentages of monocytes, eosinophils and basophils between groups. RBC and haemoglobin levels were significantly increased in the severely diseased calves compared with the healthy and moderately diseased calves.

## CONCLUSIONS

The result of this study suggests that severe BRD causes increased serum cTnI concentrations in weaned calves. Prognosis for the possible cardiac complications in calves with severe BRD may be monitored by serum cTnI measurements.

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## **KONCENTRACIJA SRČANOG TROPONINA-I KOD ZALUČENE TELADI SA RESPIRATORNIM SINDROMOM**

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Cilj studije je bio da se proceni koncentracija serumskog srčanog troponina-I (cTnI) kod zalučene teladi sa umerenim ili teškim bovinim respiratornim sindromom. Osamnaest zdravih zalučenih teladi (grupa I), 18 zalučenih teladi sa umerenim respiratornim sindromom (grupa II) i 18 zalučenih teladi sa teškim oblikom respiratornog sindroma (grupa III) je bilo uključeno u ovu studiju. Klinički znaci kao i težina respiratornog sindroma bili su određeni pomoću kliničkih indeksa. Broj krvnih ćelija, srčani biomarkeri, kao što su serumska koncentracija cTnI i kreatin-kinaza (CK-MB) i aktivnost laktat dehidrogenaze (LDH) bili su određeni u svakoj od oglednih grupa. Temperatura, frekvencija disanja i srčanog pulsa bili su signifikantno povišeni ( $P < 0,05$ ) kod teladi sa umerenim i teškim bovinim respiratornim sindromom u odnosu na zdravu telad. Vrednosti koncentracije serumskog cTnI i aktivnosti LDH bile su signifikantno povišene ( $P < 0,05$ ) kod teladi sa teškim oblikom respiratornog sindroma u odnosu na zdravu telad i telad sa umerenim oblikom bolesti. Takođe je ustanovljen signifikantan porast broja leukocita kod teladi sa umerenim i teškim respiratornim sindromom, kao i limfopenija i neutrofilija kod teladi sa teškim oblikom respiratornog sindroma. Dobijeni rezultati sugerišu da težak oblik respiratornog oboljenja dovodi do povećanja koncentracije serumskog cTnI kod zalučene teladi.